Complement factor C5 deficiency significantly delays the progression of biliary fibrosis in bile duct-ligated mice

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Abstract: Fibrogenesis represents the universal response of the liver to chronic liver injury. Complement factor C5 has been linked to fibrosis in murine toxic liver injury and human chronic hepatitis C. C5 may also play a central role in chronic cholestatic disorders, since the BA receptor FXR has been characterized as an activator of the C3 gene. We aimed to investigate, whether C5 deficiency is able to prevent biliary fibrosis in the mouse bile-duct-ligation model. BDL for 1-4weeks was performed in either Hc(0)/Hc(0) mice (deficient for C5) or WT controls. BA levels were measured by RIA. Histological examination included HE, sirius-red and immunohistochemistry. mRNA expression was quantified by RT-PCR. Protein expression levels were determined by Western blotting or ELISA. Enzymatic MMP-activity was analysed by zymography. One week BDL leads to fibrosis in WT (F2.0±0), while it is almost absent in Hc(0)/Hc(0) mice (F0.5±0.5). No differences in fibrosis can be detected at week-4. Together with delayed fibrogenesis at week-1, fibrotic markers are decreased in Hc(0)/Hc(0) mice. Expression of the inflammatory cytokine TNF- is decreased in Hc(0)/Hc(0) mice. In parallel C5 deficiency leads to an attenuated peribiliary infiltration of CD45(+) cells in fibrotic areas together with decreased MMP-9 expression and gelatinase activity. The present study proves a functional role of C5 during biliary fibrogenesis. C5 deficiency leads to attenuated inflammation and normalized MMP-9 activity concomitantly with a significant reduction of fibrosis. C5 appears to be an attractive target for future therapeutic intervention in chronic cholestatic liver disease.

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Complement factor C5 deficiency significantly delays the progression of biliary fibrosis in bile duct-ligated mice

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Abstract

Fibrogenesis represents the universal response of the liver to chronic liver injury. Complement factor C5 has been linked to fibrosis in murine toxic liver injury and human chronic hepatitis C. C5 may also play a central role in chronic cholestatic disorders, since the BA receptor FXR has been characterized as an activator of the *C3* gene. We aimed to investigate, whether C5 deficiency is able to prevent biliary fibrosis in the mouse bile-duct-ligation model.

BDL for 1-4 weeks was performed in either Hc^0/Hc^0 mice (deficient for C5) or WT controls. BA levels were measured by RIA. Histological examination included H&E, sirius-red and immunohistochemistry. mRNA expression was quantified by RT-PCR. Protein expression levels were determined by Western blotting or ELISA. Enzymatic MMP-activity was analysed by zymography.

One week BDL leads to fibrosis in WT (F2.0±0), while it is almost absent in Hc^0/Hc^0 mice (F0.5±0.5). No differences in fibrosis can be detected at week-4. Together with delayed fibrogenesis at week-1, fibrotic markers are decreased in Hc^0/Hc^0 mice. Expression of the inflammatory cytokine TNF-α is decreased in Hc^0/Hc^0 mice. In parallel C5 deficiency leads to an attenuated peribiliary infiltration of $CD45^+$ cells in fibrotic areas together with decreased MMP-9 expression and gelatinase activity.

The present study proves a functional role of C5 during biliary fibrogenesis. C5 deficiency leads to attenuated inflammation and normalized MMP-9 activity concomitantly with a significant reduction of fibrosis. C5 appears to be an attractive target for future therapeutic intervention in chronic cholestatic liver disease.

Key words

MMP9, Leukocytes, peribiliary infiltration, cholestatic liver disease.

Abbreviations

α-SMA: alpha-smooth muscle actin, BA: bile acid, BDL: bile duct ligation, Col: collagen, FXR: farnesoid X receptor, HE: Hematoxilin&Eosin, Ntcp: Na-dependent taurocholate transporter, MMP: matrix metalloproteinase, Mrp: multidrug resistance associated protein, PBC: primary biliary cirrhosis, PSC: primary sclerosing cholangitis, TGF: Transforming growth factor, TNF: tumor necrosis factor.

1. Introduction

Cholestatic liver diseases account for a substantial subset of chronic liver disease in people and are among the leading indications for liver transplantation in all age-groups [1]. In adults, PBC, PSC and cholestatic forms of hepatitis frequently progress to cirrhosis and end-stage-liver disease [2,3]. Without treatment, most patients eventually develop fibrosis and cirrhosis of the liver and may need liver transplantation in the late stage of disease [4]. Although of different etiology, the consequence of impaired bile flow in all cholestatic disorders is the retention of bile constituents including BAs. Although, recent advances have been made in the pathophysiological understanding of liver fibrogenesis [5], the development of effective therapies for chronic cholestatic disorders is still impaired by our insufficient knowledge of the molecular mechanisms by which cholestasis and retention of BAs injures the liver, and which fibrotic mechanism is involved. In chronic cholestatic disorders, T-lymphocytes and cytokines mediate persistent bile duct damage and biliary cells secrete fibrogenic mediators activating extracellular matrix formation by neighboring portal myofibroblasts [6,7]. In consequence, perisinusoidal hepatic stellate cells become activated and periportal fibrotic bands develop finally leading to end-stage cirrhosis. Irrespective of the well established cytoprotective and choleretic effects of the hydrophilic BA ursodeoxycholic acid (UDCA) no clear-cut benefit has been achieved with regard to disease progression and transplant-free survival in patients with PBC and PSC [8,9]. Recently, the side chain modified BA norUDCA has been shown to markedly improve biochemical and histological features in a mouse model of sclerosing cholangitis but clinical trials in humans are not available yet [10,11]. In the absence of an effective medical therapy, the delineation of further targets for intervention in cholestatic liver disease is urgently needed and represents the molecular basis for future treatment strategies.

It has been widely recognized that the complement system plays a critical role in the pathogenesis of a variety of chronic human disorders including chronic liver disease [12]. Activation of the complement system initiates a cascade resulting in the cleavage of the central molecule of the complement system, C3, which in turn leads to a downstream cleavage of C5. The resulting products C3a, C5a and other complement components are activators of distinct cell surface receptors translating risk signals into defined cellular responses [13].

More than two decades ago, it has been noticed that serum complement levels, particularly C3, are increased in patients with obstructive jaundice [14], PBC [15] and PSC [16]. More recent evidence suggests, that the increase of C3 in PBC patients is not due to the underlying disease entity but rather due to cholestasis in general [17]. Similar conclusions can be drawn from the description of a PSC patient with complete obstruction of the common bile duct whose serum complement C3 and C4 levels normalized upon surgical reconstitution of bile flow [18]. Intriguingly, long-term plasma exchange treatment of patients with PBC even improved liver function and decreased fibrosis marker procollagen III aminopeptide along with reduced C3 activation [19].

These clinical observations highlight the possibility of a functional link between cholestasis or BA retention and complement activation on one side, and cholestasis-induced complement activation and fibrosis progression on the other side. Indeed, regulation of complement C3 expression by the nuclear BA receptor FXR has recently been demonstrated for both human and rodent genes [20]. Given the role of the complement system in chronic toxic liver injury in mice or chronic viral hepatitis C infection in humans [21,22], and the fact that different forms of liver injury activate fibrogenesis in a disease-specific fashion [23], we aimed to investigate the particular role of BA-induced complement activation in biliary

fibrosis. Aim of this study was therefore to characterize the effects of C5-deficiency on fibrogenesis in the murine BDL model which exhibits similar structural alterations to those observed in chronic biliary obstruction in man.

2. Material and methods

2.1. Animals

8-week old C5-deficient (Hc^0/Hc^0) male mice (B10.D2-Hc0H2dH2-T18c/oSnJ) and age- and gender matched control animals (B10.D2-Hc1H2dH2-T18c/oSnJ) were purchased from Jackson Laboratory (Bar Harbor, Maine, USA) and kept under standard conditions. BDL or sham-laparatomy was performed in either C5-deficient mice (n=4, each group) or WT controls (n=5, each group) as described previously [24]. After 1 and 4 weeks the mice were sacrificed and liver tissue and blood samples were harvested. Paraffin-embedded sections from both time points were analyzed after HE and sirius-red staining for the degree of hepatic fibrosis. The staging was described previously [21]. Additionally liver fibrosis was assessed in all animals histologically by quantification of the sirius-red–positive area, using the Adobe Photoshop CS3 software. The animals received humane care and the study protocols were approved by the local Government's Animal Care Committee (Nr. 2008162).

2.2. mRNA isolation and real-time RT-PCR

Total RNA was isolated from liver by using RNeasy Mini Kit (Qiagen, Valencia, CA) according to manufacturer's instructions. mRNA was reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosytems, Mannheim, Germany). Expression was normalised against β-actin. Real-time-PCR was performed using ABI TaqMan probes on an Applied Biosystems 7900HT RT-PCR System. Taqman Probes: Ntcp: Mm00441421_m1, Mrp3: Mm00551550_m1, Mrp4: Mm01226381_m1, Col I: Mm00801666_g1, Col III: Mm01254476_m1, α-SMA: Mm00725412_s1, TGF-β: Mm03024053_m1, β6-Integrin: Mm00445326_m1, TNF-α: Mm99999068_m1, β-actin: 4352341E.

2.3. Western Blotting

Similar amounts of serum proteins were separated by SDS-PAGE, transferred to PVDF membrane and probed with C3 antibody (Santa Cruz, sc-20137, USA). After incubation with species-specific HRP-conjugated secondary antibody (Dianova, Hamburg, Germany) immune complexes were detected using ECL-PLUS detection kit (GE-Healthcare, Freiburg, Germany). Densitometric quantification of Western blots was performed using Adobe Photoshop CS3.

2.4. MMP-9 Elisa

Tissue lysates were normalized for protein concentration and equal amounts were used for ELISA. Hepatic MMP-9 protein expression was quantified by ELISA according to the manufacturer's instructions (R&D Systems, DY909).

2.5. BA quantification

Serum BAs were measured using BA RIA Kit (MP Biomedicals, Ilkirch, France) according to the manufacturer's specifications.

2.6. Gelatinase assay.

Proteolytic activity of the recombinant MMP-9 mutants was shown by gelatin zymography as described before [25]. For gelatin zymography 1mg/mL gelatin was copolymerized in a 7.5% PAGE gel and 20µg protein of pooled liver lysates of each group was loaded.

2.7. In situ zymography

An unfixed liver tissue cryoslice of 3µm thickness was stained with 1µM DAPI-solution for 3min and subsequently washed in MMP-buffer. The fluorescein conjugated DQ gelatin (Molecular Probes) was dissolved at 99°C in MMP-buffer containing 1% agarose. Using an applicator, a cover slip was coated on one side with the hot agarose buffer. After 30s cooling at room temperature the cover slip is mounted onto the tissue slice and surplus buffer was rolled out to one side. The object slides were incubated overnight at 37°C in a water

saturated atmosphere. MMP-buffer: 50mM TRIS pH7.4, 100mM NaCl, 1mM $CaCl_2$, 50µM $ZnCl_2$.

2.8. Immunohistological analysis

Immunohistology was performed as described before [26]. Antibodies used in this study: Primary antibodies were used at 2µg/ml and incubated on cryoslices over night at 4°C: Collagen I (Biodesign, T40777R), CD45 (BD, 550539), MMP-9 (R&D-Systems, AF909). Secondary antibodies: goat anti-rabbit IgG Alexa-488, goat anti-rat IgG Alexa-488, donkey anti-goat IgG Alexa-568 (Molecular probes, A11008, A11006, A11057).

2.9. Statistical analysis

Statistical significance ($P<0.05$) between respective sham-controls and BDL treated mice was determined by Mann-Whitney U-test. Data represent the mean±SEM. Analyses were performed with PASW Statistics 18.0.0, (Chicago, IL, USA) and GraphPad 5.01 (GraphPad Software Inc., CA, USA).

3. Results

3.1. Complement Factor C3 is increased in WT animals compared to C5-deficient animals

To monitor for BA retention and complement activation induced by obstructive cholestasis serum BA and serum C3 levels were measured. Compared to WT animals Hc^0/Hc^0-BDL animals represent a 194-fold, WT-BDL animals a 104-fold higher BA level, respectively (WT, 1.47±0.44µmol/L, n=5; Hc^0/Hc^0, 1.44±0.41µmol/L n=5; Hc^0/Hc^0-BDL, 389.6±35.1µmol/L, n=4; WT-BDL, 153.3±40.0µmol/L, n=5) (fig.1A). The difference between BDL animals was significant ($P<0.05$). C3 concentration was determined by Western blotting followed by densitometric analysis. Both, Hc^0/Hc^0-BDL and WT-BDL animals showed an increased complement C3 protein level compared to respective sham-controls (Hc^0/Hc^0-BDL 406±27%, WT-BDL 333±33%, each $P<0.05$)(fig.1B,C).

To confirm well established functional consequences of BA retention, we analyzed changes in the expression of BA transporters involved in the adaptation to BA overload. Basolateral Ntcp mRNA expression was significantly downregulated after 1-week of BDL in both strains (Hc^0/Hc^0 25.3±8.2% vs. WT 41.6±13.4%) (fig.1D). For the retrograde salvage transporter Mrp3, an mRNA induction in BDL mice could be observed for both Hc^0/Hc^0-BDL and WT-BDL compared to respective sham-controls (234.7±67.8% and 192±44.3%; $P<0.05$ each) (fig.1E). In parallel, Mrp4 mRNA expression was increased in BDL mice (Hc^0/Hc^0 812.1±264.4% and WT 231.3±25.6% of respective controls; $P<0.05$ each) (fig.1F).

3.2. Decreased fibrosis in C5 deficient animals with BDL

To determine whether fibrosis progression is different after BDL in Hc^0/Hc^0 compared to WT controls and sham-operated animals, respective tissue was analyzed microscopically. In Hc^0/Hc^0-BDL animals, sirius-red staining of paraffin-embedded tissue sections revealed a

lower stage of fibrosis (F 0.5±0.5 P<0.05) compared to WT-BDL animals (mean F 2.0±0). In figure 2A representative sections of controls and treated animals are shown, which clearly illustrate an absent peribiliary fibrosis which was observed in 3 out of 4 Hc^0/Hc^0 mice. In parallel a significant reduced sirius-red–positive area in histology in Hc^0/Hc^0-BDL compared to WT treated animals (111.5±46.5 vs. 316.8±110%, P<0.05) was measured (fig.2C). After 4 weeks Hc^0/Hc^0-BDL animals show a histological degree of fibrosis comparable to their WT-BDL counterparts (data not shown).

3.3. Markers for fibrosis are elevated in WT animals, but not in C5-deficient animals

To further evaluate these differences in fibrosis progression between WT and knock out animals at the molecular level, expression of a set of relevant fibrosis-related genes was analyzed at week 1.

Lower levels of collagen I and III (0,26-fold and 0.15-fold of WT-BDL, respectively; P<0.05) parallel the decreased fibrosis in Hc^0/Hc^0-BDL mice. Alpha-smooth muscle actin (α-SMA) as a marker for activated hepatic stellate cells (HSC) was significantly increased in WT-BDL animals compared to Hc^0/Hc^0 mice (3.7-fold of Hc^0/Hc^0-BDL), which show an expression comparable to WT mice (123.7±50%). mRNA expression of the profibrotic TGF-β and β6-Integrin (as part of αvβ6-Integrin) was reduced in Hc^0/Hc^0-BDL mice (0.57-fold and 0.06-fold of WT-BDL, respectively; P<0.05). In parallel to diminished profibrotic markers, the expression of proinflammatory cytokines is decreased in Hc^0/Hc^0-BDL mice (TNF-α 0.27-fold of WT-BDL) (fig.3). In line with these findings lower amounts of liver damage and infiltration of macrophages were observed in Hc^0/Hc^0-BDL mice observed in HE-staining (fig.2A).

3.4. Infiltration of leukocytes and concomitant MMP-9 increase in WT-BDL is absent in Hc^0/Hc^0-BDL

To verify inflammatory changes and fibrosis-associated events caused by BDL in the respective tissue, immunohistochemistry of frozen sections stained with pan-leukocyte marker CD45, collagen I and MMP-9 were prepared. A prominent infiltration of $CD45^+$ cells in fibrotic areas and an increased MMP-9 expression in WT-BDL animals could be monitored, while only marginal infiltration of leukocytes ($CD45^+$) and only a minor induction of MMP-9 could be observed in Hc^0/Hc^0-BDL mice (fig.4). In line with these immunohistochemical findings, MMP-9 ELISA revealed a significant quantitative increase of MMP-9 protein expression in BDL compared to untreated WT mice (166±19% of controls; $P<0.05$), while Hc^0/Hc^0-BDL showed an MMP-9 expression equivalent to all untreated or sham-operated control groups (90±17% of untreated WT controls) (fig.3B). To monitor the functional MMP-activity, gelatinase zymography have been performed. Compared to WT-BDL, Hc^0/Hc^0-BDL showed a prominently decreased gelatinase activity (fig.3C). To finally localize this enzymatic activity, in-situ zymography determined an increased gelatinase activity around the fibrotic bile ducts in WT-BDL mice (fig.3D).

4. Discussion

Common bile duct obstruction inevitably initiates the process of cholangiocyte proliferation and hepatic fibrogenesis ultimately leading to liver cirrhosis unless a reconstitution of bile flow can be facilitated either by surgery or drainage [27]. Evidence from a limited number of studies and individual observations, points to an activation of complement components, particularly C3, during obstructive cholestasis [14,17,18]. These former observations have been reproduced in a recent analysis of the biliary transcriptome in a mouse model of experimental biliary atresia where the overexpression of the C3a receptor and C1 is suggestive of the activation of the complement cascade during bile duct injury and obstruction [28]. An increased complement C3 protein level could be observed in our study in both, Hc^0/Hc^0-BDL and WT-BDL mice, which can be explained by the fact that C3 is located upstream to C5 in the complement activation cascade. This finding is well in accordance with the observed increase in serum BA levels. It has recently been shown that complement C3 expression is inducible by BAs and their nuclear receptor FXR [20]. Furthermore, BA overload causes adaptive changes in hepatocellular transporter expression as observed by decreased basolateral Ntcp [24] and the induction of the retrograde basolateral salvage transporters Mrp3 and 4 [29] which can observed in our study.

Experimental common BDL induces dynamic changes in mouse livers. In parallel to bile duct proliferation which is detectable starting from day 3 and continuously increasing until day 14 after BDL, liver fibrosis visualized by sirius-red staining is increasing from day 5 until day 14 without further deterioration thereafter [30]. In our study, bile duct-ligated wildtype mice develop fibrosis stage F2 at day 7 which is completely absent in 3 out of 4 C5-deficient mice (F0). This finding was confirmed by a reduced sirius-red–positive area in histology in Hc^0/Hc^0-BDL mice compared to WT treated animals. However, the almost absence of fibrosis in these

animals is of transient nature, since histology after 4 weeks of BDL revealed similar fibrosis stages in both C5-deficient and WT animals. The significant delay of biliary fibrosis after 1 week of BDL in C5-deficient mice was paralleled by reduced mRNA levels of fibrosis-associated genes including collagen I and collagen III, the stellate cell activation marker α-SMA, the profibrotic cytokine TGF-β, as well as β6-integrin, as part of the αvβ6-integrin [31]. Together with an increased mRNA expression of the proinflammatory cytokine TNF-α in WT-BDL animals, an excess of infiltrating leukocytes was monitored around the fibrotic bile ducts. Additionally, an increase of MMP-9 expression during chronic biliary obstruction has been described in WT livers [30] which could also be detected in our study. The immunhistological colocalization of $CD45^+$ cells and MMP-9 protein characterizes these infiltrating leukocytes as a source of MMP-9 expression. Both, $CD45^+$ cells and MMP-9 protein are clearly reduced in Hc^0/Hc^0-BDL compared to WT-BDL mice together with a decreased proteolytic gelatinase activity in the same area. Reduced peribiliary infiltration of leukocytes is consistent with a reduction of inflammatory cytokines as established activators of MMP-9 expression [32]. Decreased hepatic TNF-α mRNA expression in our study is well in accordance with the absent induction in MMP-9 expression in C5-deficient mice.

Another recent study on C5a-receptor-deficient mice demonstrated a protection against ethanol induced liver damage in a complement- and macrophage-dependent, but toll like receptor-4 independent way [33]. A reduced TNF-α expression in C5a-receptor-deficient animals in this study confirms our findings. A similar decrease in MMP-9 with antifibrotic intervention in obstructive cholestasis has been observed in the past after application of liver growth factor [34] and captopril [35].

It becomes more and more evident that the complement system plays an important pathophysiological role in chronic liver disease irrespective of the underlying mechanism. After the first publication of C5 as a modifier of fibrosis in chronic toxic liver injury in mice and chronic viral hepatitis C infection in humans [21], several reports confirmed the pathophysiological impact of the complement system on viral hepatitis C [22], non-alcoholic fatty liver disease [36] and alcoholic liver disease [37]. Genetic studies in healthy subjects have demonstrated that *C5* haplotype-tagging polymorphisms as common gene variants contribute to variations in the serum activity of the profibrogenic C5 [38]. The findings of our study hold the potential for future therapeutic intervention in patients with cholestatic liver disease without conventional treatment options. Small peptidic C5-receptor-1 antagonists have been successfully used in liver fibrosis-susceptible BALB/cJ mice to reduce hepatic collagen levels and the stage of fibrosis [21] and may be further investigated as an antifibrotic candidate drug in experimental cholestatic liver injury. As a potential option for future clinical application in liver disease, humanized anti-C5 antibodies (Eculizumab [39], Pexelizumab [40]) are available in the meantime.

In summary, our study demonstrates that C5 plays a critical role in the development of biliary fibrosis. We show that C5 deficiency results in a significantly delayed fibrosis in BDL mice. A putative mechanism for this reduced fibrosis after 1-week BDL could be the reduced inflammation, seen as attenuated peribiliary infiltration of $CD45^+$ cells in fibrotic areas, together with a decreased MMP-9 expression and gelatinase activity in Hc^0/Hc^0 mice.

Taken together C5 appears to be an attractive target for future therapeutic intervention in chronic cholestatic liver disease.

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FIGURE LEGENDS

Fig.1: Serum BAs, serum complement C3 levels and BA transporter mRNA expression.

(A) Serum BAs from WT, Hc^0/Hc^0, WT-BDL and Hc^0/Hc^0-BDL animals were measured by RIA. **(B)** Serum proteins were analysed by western blot (protein loading 25µg/lane, Coomasie gel as loading control). **C.** Densitometric analysis of serum C3 alpha chain. Relative protein expression was compared to WT. Relative mRNA quantification of BA transporters **(D)** Ntcp, **(E)** Mrp3 and **(F)** Mrp4. Asterisk represents $P<0.05$.

Fig.2: Liver histology and stages of fibrosis in Hc^0/Hc^0-BDL.

(A) Representative sirius-red stainings of liver sections from sham-control and BDL, of WT and Hc^0/Hc^0 animals and representative HE-stainings of liver sections from WT-BDL and Hc^0/Hc^0-BDL animals. Decreased histological fibrosis was observed in Hc^0/Hc^0-BDL as well as increased histological liver damage in HE-staining in WT-BDL animals (Figures show maximum of observed damages in both strains). Insert (magnification 100x) shows bile duct infarct in a higher magnification. A lower infiltration rate of immune cells was observed in Hc^0/Hc^0 animals. Scale bars: 200µm. Overlapping single pictures were taken (original magnification 50x) and remerge with Adobe Photoshop CS3 for overview. **(B)** Fibrosis. Asterisk represents $P<0.05$. **(C)** Sirius-red positive areas in %. Reduced fibrosis in Hc^0/Hc^0-BDL is confirmed by a significant decrease in sirius-red positive area compared to treated WT-BDL animals. Asterisk represents $P<0.01$.

Fig.3: Quantification fibrosis related genes and MMP-9 expression in WT-BDL and Hc^0/Hc^0-BDL mice.

(A) RT-PCR of fibrosis related genes. Col I and Col III, α-SMA, β6-Integrin, profibrotic TGF-β and proinflammatory cytokine TNF-α were analyzed. **(B)** MMP-9 Protein quantification. **(C)** Zymography of randomized control and BDL animals. Semi-quantification enzymatic activity was performed by densitometric analysis. **(D)** In situ zymography demonstrates gelatinolytic activity (green fluorescence). Bile ducts were highlighted by a dashed line bar=25µm. All sections were labelled with DNA-specific fluorochrome DAPI. Asterisk represents $P<0.05$.

Fig.4: Increased MMP-9 expression in WT animals and its absence in C5-deficient animals. Immunohistochemistry of WT-BDL and Hc^0/Hc^0-BDL mice demonstrated infiltration of MMP-9 expressing leukocytes in fibrotic areas of WT mice after BDL treatment. Staining was performed with **(A,B)** MMP-9 and COLI and **(C,D)** MMP-9 and CD-45 antibodies, respectively. Merge of the pictures shows protein colocalisation. All sections were labelled with DNA-specific fluorochrome DAPI. Scale bar: 25µm.

Figure 1

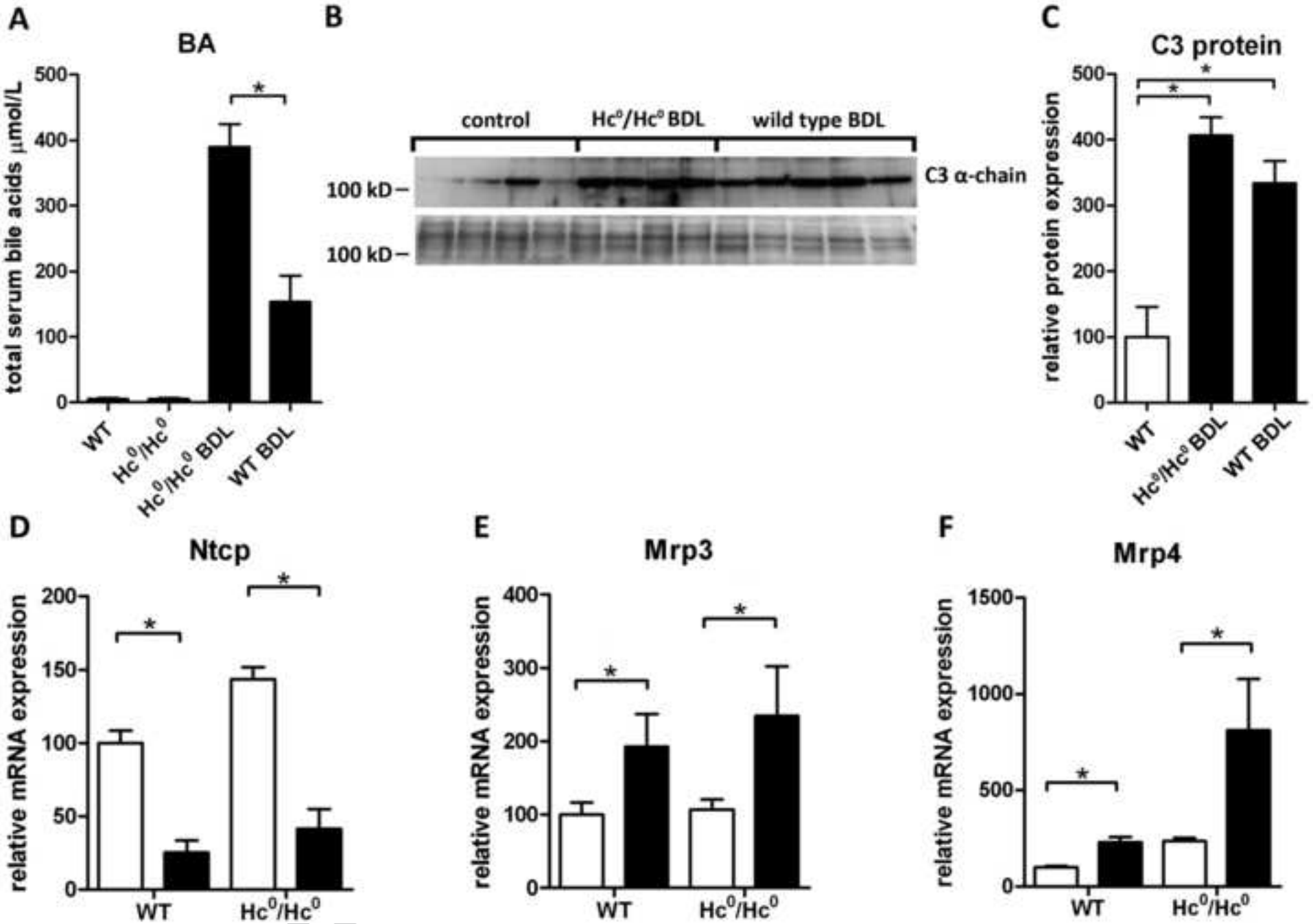


Figure 2

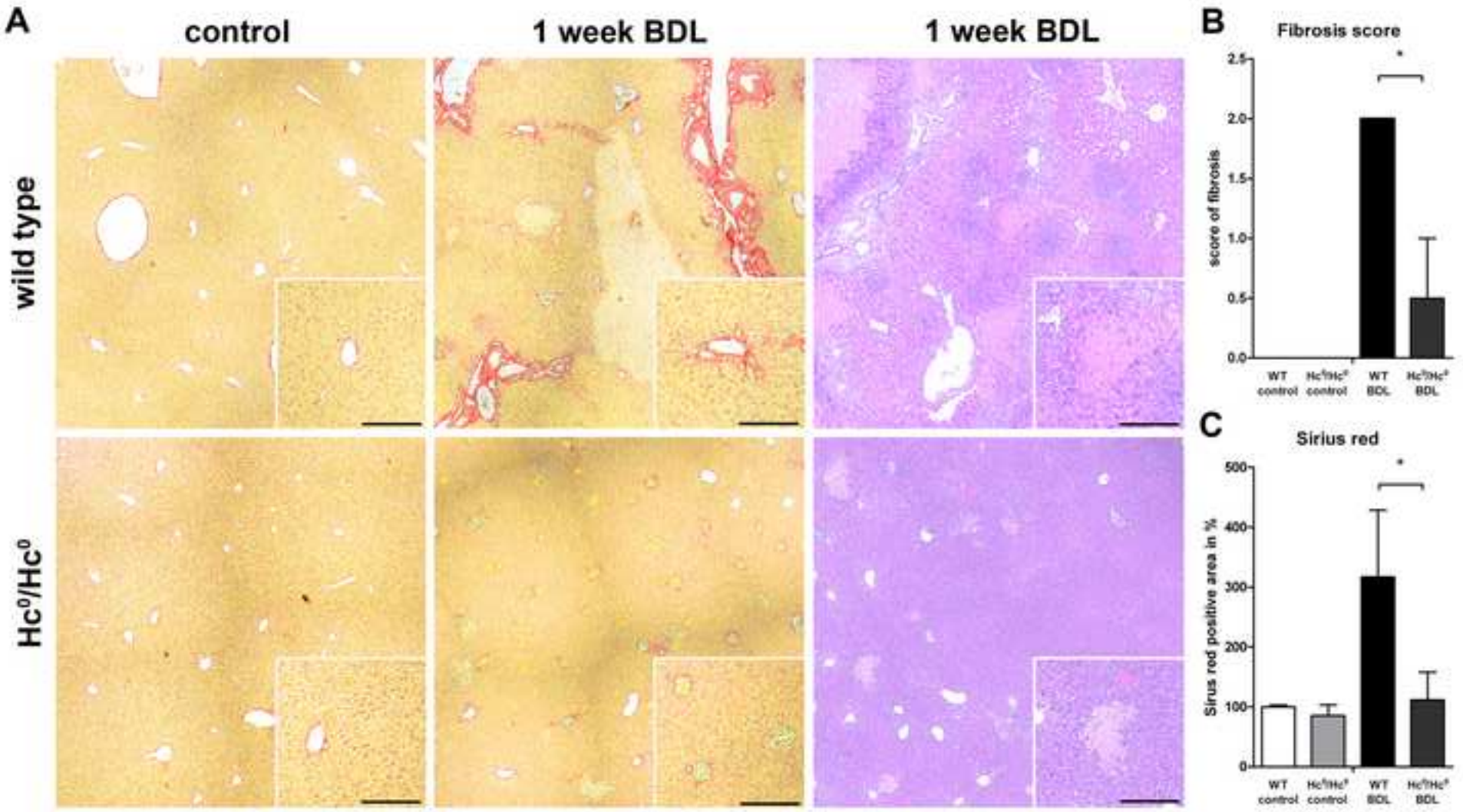


Figure 3

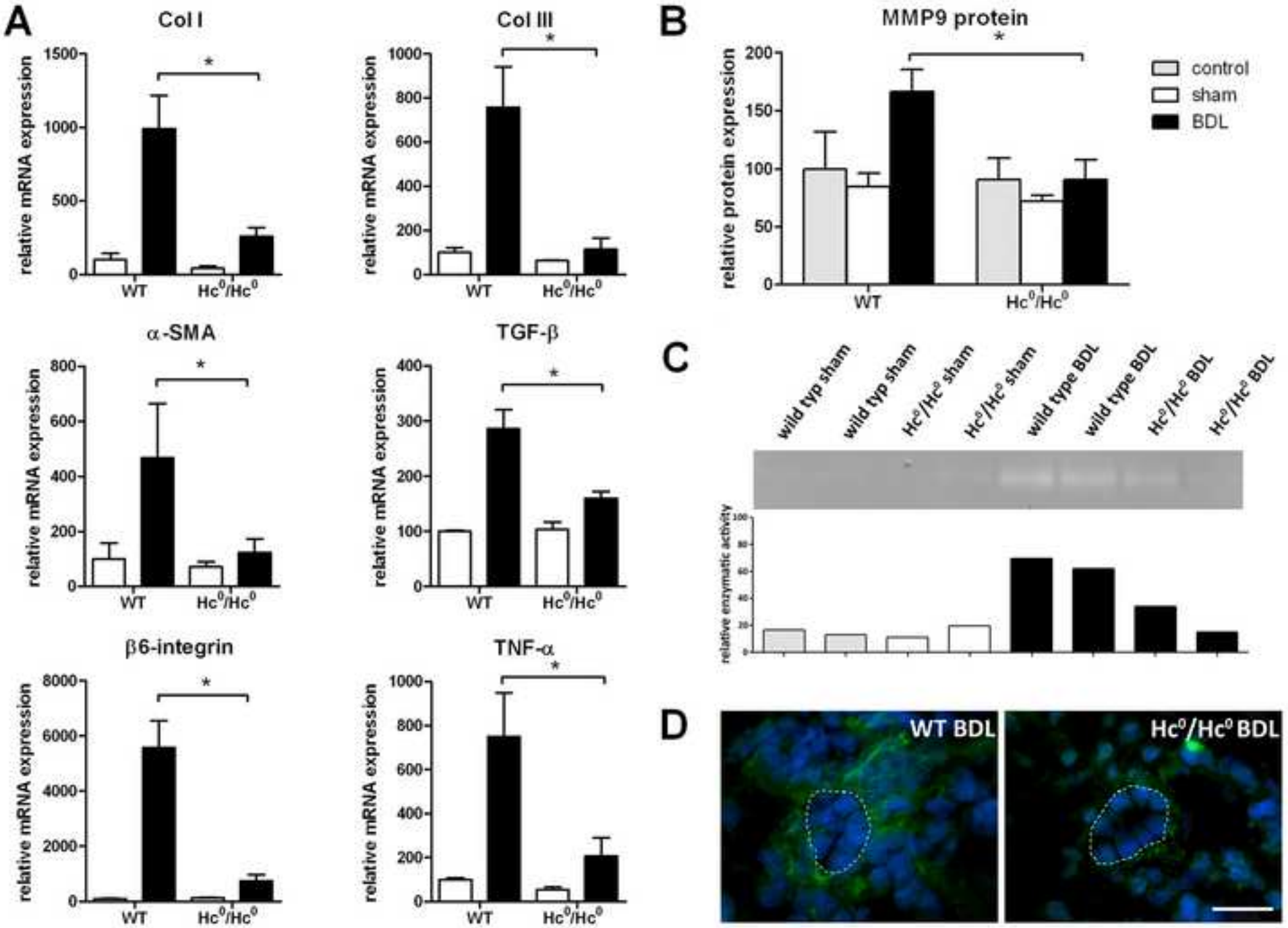
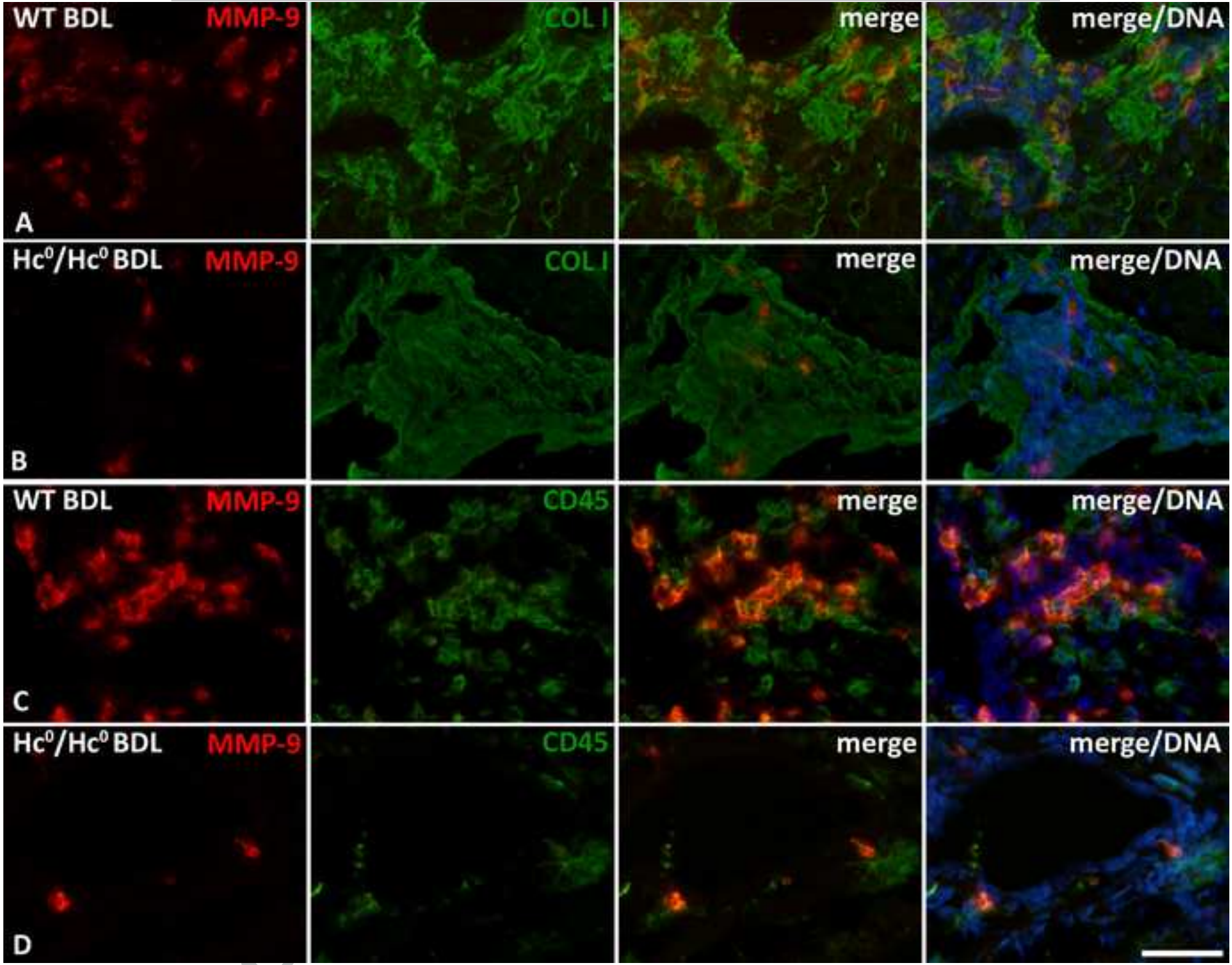


Figure 4



Highlights:

> C3 is activated during biliary obstruction in both WT and C5-deficient mice. > C5 significantly attenuates the amount of fibrosis in response upon BDL. > C5-deficient mice show decreased expression of fibrosis-associated genes and TNF-α. > Reduced peribiliary infiltration of leucocytes in fibrotic areas with C5 deficiency. > Decreased expression of MMP-9 and decreased gelatinase activity in C5-deficient mice.